



Seasonal variation in honeydew sugar content of galling aphids (Aphidoidea: Pemphigidae: Fordinae) feeding on *Pistacia*: Host ecology and aphid physiology

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Summary

We investigated the possibility that seasonal variation in host-tree sap quality was reflected in aphid honeydew sugar content. Aphids (Homoptera) feed on the phloem sap of their host plants and excrete sugar-rich honeydew. We compared the sugar composition of honeydew excreted by four species of closely related aphids (Pemphigidae: Fordinae) inducing galls on *Pistacia palaestina* (Anacardiaceae). Samples were collected four times a year in 1997 and 1998. Samples from one species feeding on the roots of a secondary host, a perennial herb, were also included in the study.

More than 20 sugars were detected in the honeydew. Sugars that were present in more than 40% of the samples were analyzed quantitatively in a hierarchical manner. The mean proportions of each sugar of the total sugar content in different species were not significantly different, but samples taken at different dates contained significantly different proportions of the sugars.

The most frequent sugars in all species were glucose and fructose. Generally, the proportion of glucose exceeded fructose, but in honeydew from aphids feeding on the roots of the secondary host the reverse was true. We suggest possible explanations for the observed patterns, and discuss a possible contribution of Fordinae honeydew to the food web in the micro-ecosystem.

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Zusammenfassung

Wir untersuchten die Möglichkeit, dass sich die saisonale Variation der Saftqualität des Wirtsbaumes im Zuckergehalt des Honigtaus der Aphiden widerspiegelt. Die Aphiden (Homoptera) ernähren sich vom Phloemsaft der Wirtspflanzen und scheiden zuckerreichen Honigtau aus. Wir verglichen die Zuckerzusammensetzung des Honigtaus von vier Arten nahverwandter Aphiden (Pemphigidae: Fordinae), die Gallen auf *Pistacia palaestina* (Anacardiaceae) induzieren. Die Proben wurden viermal jährlich 1997 und 1998 gesammelt. Darüber hinaus wurden Proben einer Art in die Untersuchung mit einbezogen, die sich auf den Wurzeln eines Sekundärwirtes, eines perennierenden Krautes, ernährt. Es wurden mehr als 20 Zucker im Honigtau festgestellt. Die Zucker, die in mehr als 40% der Proben vorkamen, wurden quantitativ in hierarchischer Weise analysiert. Die mittleren Anteile eines jeden Zuckers am Gesamtzuckergehalt der unterschiedlichen Arten waren nicht signifikant verschieden, aber die Proben, die zu unterschiedlichen Daten genommen wurden, enthielten signifikant unterschiedliche Anteile der Zucker. Die häufigsten Zucker bei allen Arten waren Glukose und Fruktose. Im Allgemeinen war der Anteil der Glukose größer als der der Fruktose. Im Honigtau der Aphiden, die sich auf den Wurzeln des Sekundärwirtes ernährten, war jedoch das Gegenteil zutreffend. Wir schlagen Erklärungen für die beobachteten Muster vor und diskutieren einen möglichen Beitrag der Fordinae zum Nahrungsnetz im Mikro-Ökosystem.

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Introduction

That insect herbivores are affected by seasonal changes in the phenology of their host plants is well documented. In particular, gall-inducing insects—like cecidomyiid midges and gall-inducing aphids—must adjust their hatching times with the bud-break phenology of their host trees, because galls can only be induced in the narrow time-window when the shoots grow (Yukawa, 2000). Parry (1978) suggested that the amino-acid content of spruce trees changed with the season, and that seasonal soluble nitrogen levels of the sap were probably associated with nymphal mortality and morph determination of *Adelges cooleyi*, a gall-inducing Adelgid on Douglas fir (Parry, 1978). Recent research on the spruce aphid, *Elatobium abietum*, suggests that seasonal changes of the amino-acid concentration in the needle sap affected aphid growth rate and final size, and was correlated with (but not necessarily the cause of) the production of alates (Day, Armour, & Docherty, 2004). Sucking insects, like aphids, must be able to detect seasonal changes in sap quality of the trees when leaf senescence at the end of summer is a prelude to leaf fall. Sequeira and Dixon (1997) suggested that seasonality in host plant sap quality is involved in the population dynamics of the turkey-oak aphid, *Myzocallis boerneri*.

An indirect approach to the study of seasonal changes in sap quality may be the analysis of the composition of aphid honeydew. Aphids produce large quantities of sugar-rich honeydew while

feeding on the phloem of their host plants. Plant sap contains amino acids and secondary plant products in small quantities (Molyneux, Campbell, & Dreyer, 1990). The importance of the amino acids, particularly those supplied by symbiotic bacteria, for aphid nutrition and survival has been studied intensively in experiments with aposymbiotic aphids (e.g., Wilkinson, Ashford, Pritchard, & Douglas, 1997) and is addressed in many textbooks on aphid biology (e.g., Dixon, 1998).

A common interpretation of the excretion of aphid honeydew is that the aphids must ingest large quantities of sugar-rich sap to extract the necessary amino acids they need for growth and reproduction, and the surplus sugars are excreted. Much of the literature on aphid honeydew deals, accordingly, with the amino acid content of the aphid diet (a recent example: Yao & Akimoto, 2002). The need for amino acids may not be the only explanation of honeydew excretion. Symbiotic bacteria supply the aphids and whiteflies with essential amino acids, which may be missing in the plant sap (e.g., Sandström & Moran, 1999)—and aphid infestation may enhance the supply of these components to the aphids: aphid-infested leaves contain more free amino acids than uninfested leaves (Sandström, 2000).

The amino-acid contents of the phloem sap of the same plant may vary at different times of the day (Hendrix & Salvucci, 1998) or in different parts of the plant (Fisher, 1983, 1987; Fisher & Gifford, 1986). It is quite difficult to extract pure phloem sap from a plant except by the severed mouthparts

of a plant bug or an aphid (stylectomy). (Fisher & Frame, 1984; Sandström & Moran, 1999). Exudates extracted from the same host (barley) by different species of aphids differed in composition and concentration of amino acids (Sandström, Telang, & Moran, 2000).

By far the most abundant component of plant sap is sucrose (See Appendix III in Zimmermann & Ziegler, 1975). A growing number of recent publications on aphid nutrition focus on understanding the fate of sucrose ingested by the aphids. Most of this research is done on aphids and whiteflies fed artificial, chemically defined diets (e.g., Rhodes, Croghan, & Dixon, 1996, 1997; Febvay, Rahbe, Rynkiewicz, Guillaud, & Bonnot, 1999; Ashford, Smith, & Douglas, 2000). Consequently, most of the information concerns a few species that can be reared in the laboratory—mainly the pea aphid *Acyrtosiphon pisum* and the silverleaf whitefly *Bemisia argentifolii* (Hendrix, Wei, & Leggett, 1992; Salvucci, Wolfe, & Hendrix, 1997).

Most studies detected little or no sucrose in the honeydew, but found oligo- and polysaccharides in considerable quantities. The insects seem to convert sucrose—or its monosaccharide derivatives—into oligosaccharides of different sizes. A possible explanation for this is the need to avoid dehydration, since the osmotic pressure in the diet is higher than that of the haemolymph (Fisher, Wright, & Mittler, 1984; Rhodes et al., 1996, 1997; Febvay et al., 1999; Wilkinson et al., 1997; Salvucci et al., 1997; Ashford et al., 2000). There is evidence that the chemical rearrangement of sucrose and its derivatives for the synthesis of oligosaccharides is carried out by the bacterial symbionts of the aphids and whiteflies (Davidson, Segura, Steele, & Hendrix, 1994; Febvay et al., 1999).

Different species of aphids sharing the same host are likely to utilize the same phloem sap. Differences in sugar composition of the honeydew may result from different metabolic rates in the aphids. Völkl, Woodring, Fischer, Lorenz, and Hoffmann (1999) reported that the honeydew produced by four aphid species feeding on cloned individuals of the same plant, was both quantitatively and qualitatively different in sugar composition.

In Israel, about 15 species of aphids (Fordinae, Pemphigidae) induce galls on three common tree species of the genus *Pistacia* (Anacardiaceae) (Koach & Wool, 1977; Wool, 1995). Two or more of these species are often found on the same shoot or even on the same leaf of their host plant (Inbar & Wool, 1995). Each species induces a characteristic gall. Each gall is induced by a single fundatrix and may contain hundreds or even thousands of her parthenogenetic offspring, constituting a clone.

The life cycle of these aphids involves host alternation between *Pistacia* and the roots of secondary hosts (grasses) where the aphids reproduce in winter without inducing galls (review in Wool, 1984, 2004; Blackman & Eastop, 1994). The galls are sinks for sugars produced by photosynthesis, and may import them from sources close to the gall or far from it depending on sink strength (Burstein, Wool, & Eshel, 1994).

Unlike free-living aphids, which may migrate to a new feeding site on a new leaf or shoot, galling aphids stay at the same spot throughout the season. Seasonal changes in the sap contents should be pronounced in a deciduous tree like *Pistacia*. As the tree comes out of dormancy in the spring, rapid growth of new shoots continues for a few weeks—a time window for the aphids to induce their galls. Later the leaves visibly change. At the end of summer, changes in leaf phenology are particularly obvious to the human eye—as the tree approaches leaf abscission. This must be noticed by the aphids in the galls, which then produce rapidly the final offspring generation, a prelude to alate formation and evacuation of the galls before leaf fall (our research reveals that the direct trigger for alate formation is aphid density (Wool & Ben-Zvi, 1998). Changes in tree sap sugar content may be the result of altered photosynthetic rates as the leaves get ready to drop. Such changes may be reflected in the honeydew.

In the galls, the honeydew often accumulates in the form of small, bead-like wax-coated droplets (Pers. Obs.), as described in detail in *Pemphigus spyrothecae* (Pike, Richard, Foster, & Mahadevan, 2002). In species of *Pemphigus*, where the gall has a permanent opening, groups of aphids have been observed to remove the wax-coated honeydew, a behavior interpreted as a primary stage of sociality (Benton & Foster, 1992). This behavior was not observed in the Fordinae (and is not possible in the closed galls of some species). The accumulated honeydew is released when the galls open in the autumn.

Considerable research in the late 1990s (Stadler & Müller, 1996; Stadler, Michalzik, & Müller, 1998; Stadler, Solinger, & Michalzik, 2001) found little evidence in support of the claim that aphid honeydew contributes to the ecology of the host trees. We suggest that the honeydew of the Fordinae, packed in wax as it is, may have a role—however small—in the food web of the microecosystem.

Our purpose was to study the temporal patterns in composition of the honeydew of different species of Fordinae living on the same host. No previous information on the honeydew of the Pemphiginae is

available, and no non-galling aphids are known to us that feed naturally on *Pistacia* in Israel. We sought answers to the following questions:

- 1) How similar is the honeydew sugar content excreted by different galling-aphid species feeding on the same host?
- 2) Are seasonal changes in the sap of the host tree reflected in the composition of aphid honeydew?
- 3) What, if any is the chance that the honeydew released from the galls contributes to the energy flow in the micro-ecosystem?

Materials and methods

Aphids

Galls of four species were collected on *Pistacia palaestina* trees, at our study site along the Beit Shemesh–Beit Guvrin road, about 40 km south-east of Tel Aviv (Israel Grid 148 128): *Baizongia pistaciae* (L.) induces large, horn-shaped galls, generally on the apical bud of a shoot. Galls may contain several thousand aphids (Wertheim, 1954; Wool, 2002). *Geioica wertheimae* Brown & Blackman induces marble-shaped galls near the leaflet midvein. These galls may contain up to 1000 aphids (Wool & Ben-Zvi, 1998). *Forda formicaria* von Heyden (Wool & Bar-El, 1995), and *F. marginata* Koch final galls are formed on leaflet margins and may contain 100–150 aphids. Photographs of the galls are provided by Koach and Wool (1977) and Wool (2004). Two or more species may be found on the same shoot, and the latter 3 may share the same leaf (Inbar & Wool, 1995). *G. wertheimae* and *F. formicaria* may occasionally share the same leaflet, and compete for nutrients. When this happens, the latter species usually dies (Inbar, Eshel, & Wool, 1995).

Galls were collected in June, August, September and October 1997, and in June, July and October 1998 (in October, leaf abscission was well under way and many galls already released their aphids). In the summer of 1998, we collected root-inhabiting aphids (*F. formicaria*) on the perennial grass, *Oryzopsis miliacea*.

Honeydew collection

Honeydew from a feeding aphid colony is usually collected by placing filter paper or aluminum foil under the colony. This is not possible in aphids living in closed galls. The aphids we collected did not excrete honeydew without tactile stimulation.

The galls were opened in the laboratory and individual aphids were stimulated to produce honeydew by gently stroking their backs with a thin brush (we had watched ants (*Monomorium pharoni*) “milking” aphids in root cages, by drumming with their antennae on the aphid’s abdomen, and tried to imitate this stimulus). Honeydew from root-feeding *F. formicaria* was collected in the same way.

The honeydew droplets were collected on small pieces of aluminum foil, air dried, and mailed for analysis to the Western Cotton Research Laboratory in Phoenix, AZ, which is specialized in honeydew analysis (Hendrix et al., 1992; Hendrix & Wei, 1994).

Analytical methods

Foils were washed with ca. 10 ml of hot (80 °C) deionized water. The water was then removed by lyophilization and the resulting sugar suspended in 200 µl of water for sugar analysis by anion high-performance liquid chromatography (HPLC). Sugars were separated using two Dionex PA-1 columns connected in series, and an elutant of 0.2 M NaOH in which a sigmoidal gradient of 0 to 0.5 M sodium acetate was introduced at sample injection (Hendrix & Wei, 1994). Sugars and polyols were detected by a Dionex-pulsed amperometric detector connected to a computer which calculated the area under each peak in the resulting chromatograph.

The output of each run of ten samples was accompanied by a reference chromatograph in which known sugars were run as standards. The peaks in the standard chromatograph were numbered and identified. In the sample curves, sugars were identified and quantified by their retention time (RT) and their relative peak areas in comparisons with the standards.

The sugar content in some of the early samples was too low to be analyzed, even though the analytical technique was capable of detecting sugars and polyols in quantities as small as 100 ng. This made it necessary to pool all droplets excreted by individual aphids from the same gall and analyze them as one sample. The biological justification for doing so is that all aphids within a gall are genetically identical, and the whole clone can be considered one organism.

The small quantities of honeydew per sample precluded the use of further tests (such as in Hendrix & Salvucci, 1998) to confirm the identity of the sugars.

Data analysis

The sugars in our samples were eluted in less than 20 min. We assembled all peaks in a frequency distribution with a class interval of 0.5 min. Peaks falling into the same RT class were considered identical sugars. For each species, we listed all sugars according to their RT and noted the frequency of samples in which each sugar was present. Since the absence of a sugar from a small sample may be accidental, we selected for detailed analysis those sugars which were present in at least 40% of the samples for that species.

The proportion of each sugar of the total quantity of sugars in the sample (obtained by integration of the area under the peak) was used in the quantitative analysis. The mean percentages of the sugars were compared among aphid species and sampling dates.

We analyzed the data quantitatively in a hierarchical manner, and tested for differences in content of the sugars among species and among samples of the same species collected at different times of the year (sampling dates were not the same for different species and were considered a random effect). The statistical analysis was carried out using the BiomStat program package, version 3.3 (Rohlf & Slice, 1999) which can handle "nested" analysis of variance with unequal sample sizes and makes all the necessary adjustments. The percentages were angular-transformed to ensure normality (Sokal & Rohlf, 1995).

We collected 58 samples of *Baizongia pistaciae*, 25 of *G. wertheimae*, 13 of *F. formicaria*, 8 of *F. marginata*, and 21 from aphids (*F. formicaria*) from roots of the secondary host. The number of samples actually analyzed varied among sugars, because not all sugars were detectable in a given sample.

Results

More than 20 sugars were detected in the honeydew of each species. Most—but not all—of the sugars occurred in the honeydew of all species, in varying proportions (an example of this variation is illustrated in Fig. 1). One unidentified sugar, eluted at RT = 5–5.5 min, was detected in 19 of the 58 samples of *Baizongia pistaciae* but in no other species. (The number of samples from other species was much smaller, and we cannot be sure that we have not missed it accidentally.)

The most frequent sugars were not necessarily present in the highest concentration. The concentrations and tentative identification of the more frequent sugars (based on comparisons with the standards) are listed in Table 1 by their RT.

Glucose and fructose were major components of the honeydew of all species, and were eluted in our system between RT = 6–6.5 and 6.5–7 min, respectively (Table 1).

In *Baizongia pistaciae*, the mean percent glucose was larger than fructose in the same samples but the difference was not significant. There was no correlation between the percentages of the two sugars in the same samples ($r = 0.118$, $df = 47$; $p = 0.42$). Correlation calculated on angular transforms of the percentages (Sokal & Rohlf, 1995).

In *G. wertheimae*, the mean proportion of glucose was twice that of fructose, but the negative correlation between the sugars was not significant ($r = -0.247$, $df = 14$; $p = 0.356$). In the few samples of *F. marginata*, and in *F. formicaria*, the mean percent glucose was also twice that of fructose. Interestingly, in samples of the latter species, collected from the roots of the perennial grass *Oryzopsis miliacea*, the pattern was reversed

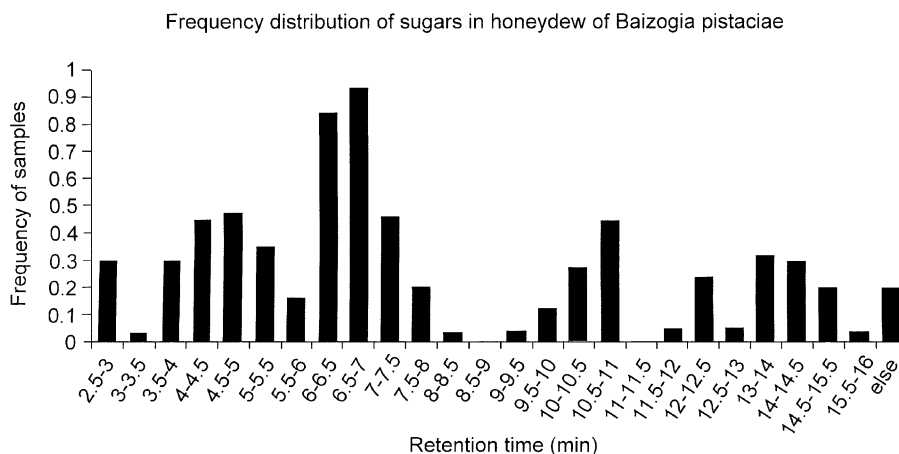


Figure 1. Frequency distribution of sugars in 58 samples of honeydew from *Baizongia pistaciae*. The most frequent sugars were glucose (RT = 6–6.5 min) and fructose (RT = 6.5–7 min).

Table 1. Sugar content of honeydew of galling aphids on *Pistacia palaestina* (percent of total sugars in a sample \pm SE)

RT (min)	Sugar	<i>B. pistaciae</i> 58	<i>G. wertheimae</i> 25	<i>F. marginata</i> 8	<i>F. formicaria</i> 13	Roots 21
< 4	Inositol?	29.9 \pm 4.99 (17)	26.6 \pm 5.11 (13)	(1)	18.2 \pm 6.15 (7)	9.2 (2)
4–4.5	Mannitol?	17.6 \pm 3.38 (22)	18.2 \pm 4.19 (10)	(1)	1.2 \pm 0.36 (4)	20.9 \pm 7.11 (11)
4.4–5	Trehalose	26.7 \pm 3.97 (27)	22.7 \pm 6.46 (9)	12.8 \pm 2.35 (4)	0.8 (2)	3.8(3)
5–5.5	?	12.6 \pm 2.85 (19)				
6–6.5	Glucose	25.6 \pm 2.19 (49)	30.0 \pm 4.79 (25)	26.6 \pm 8.30 (6)	23.5 \pm 4.84 (10)	15.8 \pm 1.62 (20)
6.5–7	Fructose	21.1 \pm 2.32 (52)	17.3 \pm 2.98 (19)	13.3 \pm 3.30 (7)	14.3 \pm 4.37 (11)	26.5 \pm 4.23 (17)
7–7.5	?	11.5 \pm 4.26 (20)	6.4 \pm 1.72 (20)	6.6 (3)	6.7 \pm 1.64 (6)	19.6 \pm 4.25 (10)
10.5–11	Sucrose?	9.7 \pm 4.26 (15)		14.5 \pm 5.36 (4)	13.4 \pm 6.00 (11)	4.9 \pm 1.21 (10)

The numbers of samples collected are listed above the columns. (n) = the numbers of samples containing each sugar (frequency). Sugars are listed in order of elution. Values for $n = 1$ and SE for $n < 4$ were omitted.

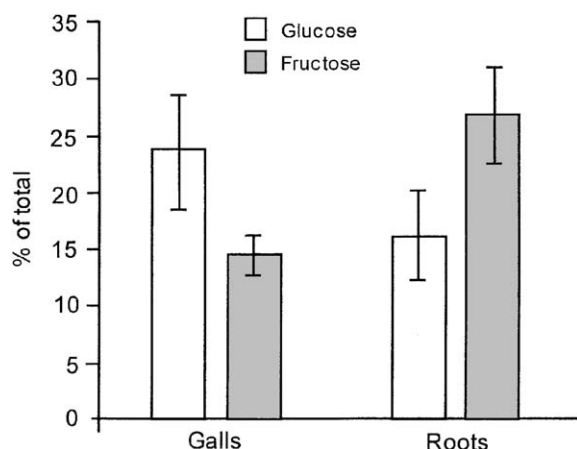


Figure 2. Glucose and fructose content in samples of *Forda formicaria* feeding in galls of *Pistacia palaestina* and on roots of *Oryzopsis miliacea*. Note that the relative proportions of the two sugars are reversed in the root-feeding aphids.

(Table 1; Fig. 2). We found no other species on grass roots during the study, so we cannot tell if this pattern is general or specific to *F. formicaria*.

Sugar alcohols—tentatively identified as inositol (eluted at RT = 3–4 min) and mannitol (RT = 4–4.5 min.) were the first sugars to be eluted from the columns. A peak at 4.5–5 min, was tentatively identified as the disaccharide trehalose. These sugars were less frequent than glucose and fructose and were present in fewer than half the samples, although the fraction they constituted of the total sugars in some samples were considerable (Table 1).

In some samples there were clear peaks eluted at RT = 10 min or more. They constituted small fractions of total sugars (generally less than 5%) but there were some exceptionally high values, up to

20% in one sample. These sugars were not identified with certainty, but those with RT = 10.5–11 min. were probably sucrose, while those with RT = 12–15.5 min were other oligoaccharides or sugar-phosphates.

Quantitative analysis

Figure 3 illustrates mean percentages (\pm SE) of glucose and fructose in the honeydew of different species. Some differences seem substantial, although the standard errors were quite large. However, the samples were taken at different times of the year, and the species means illustrated in the figure may be confounded by differences among sampling dates. This led us to a quantitative hierarchical analysis of the sugar concentration.

Nested ANOVA indicated that the differences among species in the proportions of the common sugars were not significant (Table 2). The added variance component due to species was very small (see bottom of the table). Differences among sampling dates accounted for a large proportion of the variation (illustrated in Fig. 4 for *Baizongia pistacia* as an example).

We compared the temporal patterns of mean percentages of several sugars among different species. There was an increase in the percentage of monosaccharides (glucose, fructose and an unidentified sugar with RT 7–7.5, which may be a derivative of fructose) in the honeydew from early June to July, remaining high in later months (Table 3). The similarity of the patterns in different species suggests a response to variation in the ingested phloem sap.

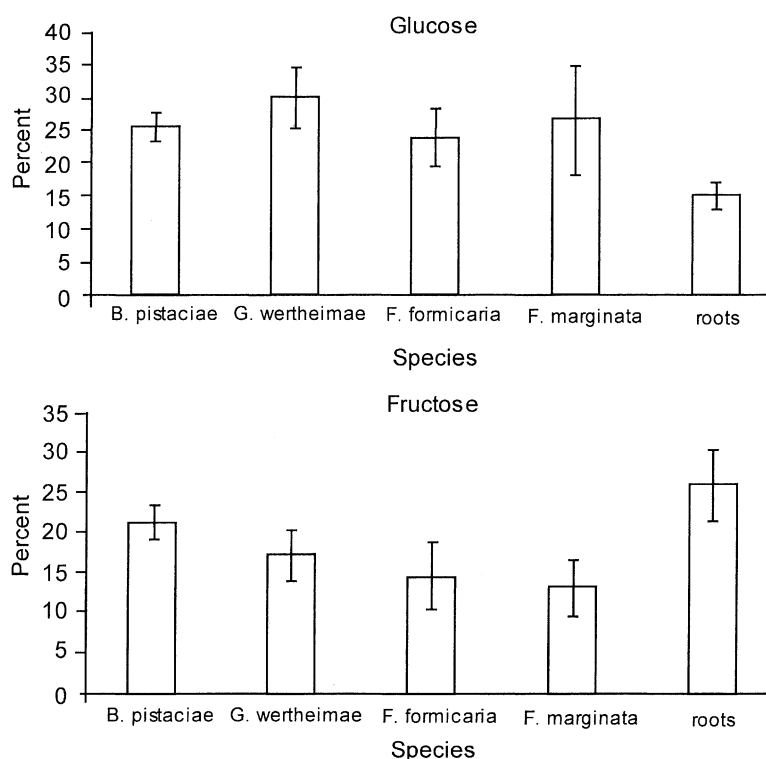


Figure 3. Glucose and fructose content in honeydew of different species of Fordinae, averaged over all sampling dates (means \pm SE). The differences among species were not significant statistically (see text).

Discussion

Honeydew in galling aphids

In this study we encountered difficult technical problems which do not occur in analyses of honeydew in free-feeding aphids. Honeydew from a feeding colony is normally collected by placing a piece of foil or paper under the branch or leaf. In our galling aphids this is not possible. We are aware that disconnecting the gall from the branch may affect the transport of phloem sap to the aphid colony. Also, the honeydew in the galls is normally enclosed in wax as it leaves the anus, and stored as "liquid marbles" (Pike et al., 2002), while we collected it before it was waxed—but to do so we had to cut open the galls and induce the aphids to excrete. Until more work is done on the honeydew of galling aphids, we have no way of knowing what effects these procedures had on aphid metabolism.

The major component of the phloem sap of plants is sucrose (reviewed by Zimmermann and Milburn, 1975). The one species of *Pistacia* listed in the review, *Pistacia lentiscus*, is no exception (see also Febvay et al., 1999; Ashford et al., 2000). However, very little sucrose was found in honeydew from aphids and whiteflies, probably due to the

presence of sucrase in the insect (Salvucci et al., 1997). Experiments with the aphid *Acyrtosiphon pisum* on artificial diets revealed that at low dietary sucrose (<15%) the honeydew contained mainly mono- and disaccharides. When dietary sucrose exceeded 15%, the honeydew was dominated by oligosaccharides (Rhodes et al., 1997). This evidence supports the opinion that honeydew is not a simple excretion of surplus sugars, but rather that the sap is metabolized by the insects, and the output may reflect their metabolic needs (Rhodes et al., 1996).

We compared the composition of the honeydew excreted by four species of Fordinae feeding in galls on the same species of primary host. We did find a peak tentatively identified as sucrose in our honeydew samples. We may perhaps infer from these data that *Pistacia palaestina* sap contains less than 15% sucrose since monosaccharides were dominant in the aphid honeydew (the sap of *Pistacia lentiscus*, as listed in Zimmermann and Milburn (1975), contained 10–20% sucrose).

In the honeydew sampled from galls of all Fordinae in our study, glucose concentration was higher than fructose (Table 1). In whiteflies, fructose absorbed from the gut is converted to the polyol sorbitol, but glucose is not (Hendrix &

Table 2. "Nested" ANOVA on angular transforms of percentages of some common sugars in honeydew of Fordinae aphids feeding on *Pistacia palaestina*

Sugar	Level	MS	df	F	p
Glucose RT = 6–6.5	Species	267.76	4	0.509	0.730 ns
	Dates	525.84	14	7.230	<0.001
	Within	72.70	86		
Fructose RT = 6.5–7	Species	214.22	4	1.150	0.366 ns
	Dates	186.10	17	1.570	0.091 ns
	Within	118.58	85		
Trehalose RT = 4.5–5	Species	31.61	1	0.560	0.820 ns
	Dates	565.99	7	4.740	0.0015
	Within	119.39	26		
Mannitol? RT = 4–4.5	Species	397.18	3	2.730	0.114 ns
	Dates	145.65	8	0.900	0.528 ns
	Within	161.92	35		
Inositol? RT < 4	Species	1458.60	4	0.888	0.524 ns
	Dates	1642.40	6	7.440	<0.001
	Within	220.70	34		
Oligo- Saccharides RT > 11	Species	1069.90	4	1.403	0.287 ns
	Dates	762.30	13	4.020	<0.001
	Within	189.50	64		

Variance components (percent) among

Sugar	Species	Dates	Within
Glucose	0	53.70	46.30
Fructose	1.140	10.58	88.29
Inositol	0	66.06	33.94
Oligosaccharides	4.500	39.55	55.95

The *F*-values were adjusted for unequal samples sizes (Sokal & Rohlf, 1995). Each sugar was analyzed separately.

Salvucci, 1998). In *Acyrtosiphon pisum*, experiments with differentially labeled dietary sucrose showed that the fructose moiety of sucrose seems to be very efficiently and preferentially respired by the aphid, while the glucose moiety is incorporated into oligosaccharides (Ashford et al., 2000). Similar mechanisms may be the cause of the excess of glucose in the honeydew of the Fordinae.

Völkl et al. (1999) reported that four species of aphids feeding on the same hosts excreted very different sugar concentrations in their honeydew, reflected also in ant attendance (these differences may be attributed to differential ability of the aphids to transform the ingested sucrose). We found no significant differences among species, and have no evidence that feeding at different sites on the tree affects the sugar composition in the honeydew. On the other hand, we did find significant differences in sugar content among samples taken at different times of the year. These differences could be due to changes in the sugar content of the host-plant phloem sap at different

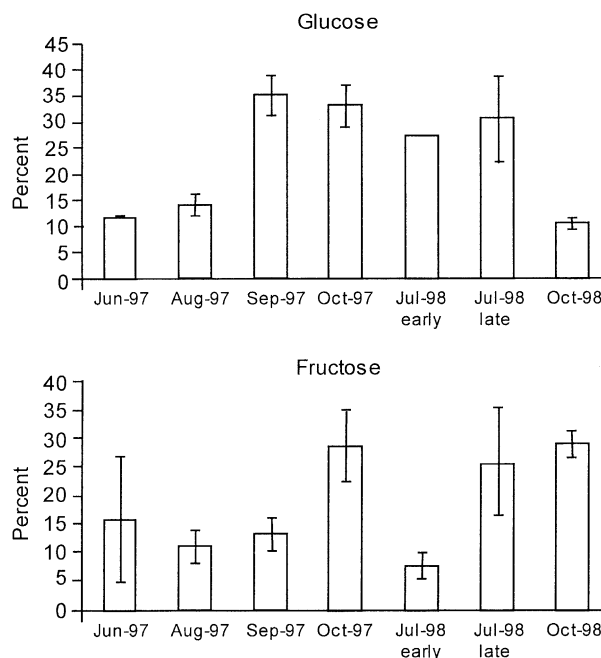


Figure 4. Mean percentages (\pm SE) of glucose and fructose in samples of *B. pistaciae* collected at different times of the year.

times of the year. Alternatively, aphid metabolism may also change seasonally. In a detailed study of the honeydew of the aphid *Tuberculatus quercicola* (not a gall former), on oak in Japan, the amino acid content of the host phloem sap changed greatly as the summer progressed, but the composition of the honeydew remained the same (Yao & Akimoto, 2002). Metabolism of dietary sugars to CO_2 is temperature-dependent (Salvucci & Crafts-Brandner, 2000), and could also be affected by changes in the water balance in the hot and dry Mediterranean climate of Israel, in particular in late summer (August–October), as the trees approach leaf abscission.

Alternative explanations may be offered for the difference of the relative proportions of glucose and fructose in honeydew of the gall-feeding and root-feeding *F. formicaria*. The aphids at the two stages feed on different plants: the sap of the perennial grass *O. miliacea* may be quite different in composition from the leaves of the tree *Pistacia palaestina*. The aphids at the different stages in the life cycle are very different in size, color and morphology. Moreover, the root aphids feed during the cool and wet winter and spring, the gall aphids during the hot and dry summer. At the present stage of our knowledge, it is difficult to know which is the more likely explanation.

Table 3. Mean percentage of some sugars in the honeydew of species of Fordinae feeding on the same *P. palaestina* trees at different months

Species	June (early)	June (late)	July	Aug.	Sept.	Oct.
<i>Baizongia pistaciae</i>	15.9	27.9	30.0	25.6	56.4	64.9
<i>G. wertheimae</i>	25.2	43.9	55.2	37.3	67.4	29.1
<i>F. marginata</i>	10.3	35.2				
<i>F. formicaria</i>	40.1	56.6	32.7	20.2		

Ecological implications

Apart from its interest for aphid biologists, aphid honeydew may have a wider role in the economy of the habitat. Honeydew is further consumed by ants, flies, wasps, and other insects (Darwin wrote in the Origin: "One of the strongest instances of an animal apparently performing an action for the sole good of another, with which I am acquainted, is that of Aphides (sic) voluntarily yielding ... their sweet secretions to ants". Darwin, 1898 (Origin of Species, 6th ed., pp. 193–194). Owen and Weigert (1976) suggested that when honeydew is produced in large quantities, it may be washed by the rain and filter down to the soil and become food for microorganisms, thereby positively affecting the food network and forest ecology. This suggestion was criticized for ignoring the drain in tree resources caused by aphid feeding, but it stimulated detailed investigations in search of evidence (Stadler & Müller, 1996; Stadler et al., 1998, 2001). These investigations showed that honeydew is metabolized by bacteria and fungi on the leaves (the phyllosphere), in particular in coniferous forests, and contributes significantly to the growth of bacterial populations in particular in months of peak aphid abundance, but the impact is reduced on the way down and no differences were found in carbon or nitrogen content of the soil under aphid-infested and uninfested trees.

Galling aphids seem to excrete rather limited amounts of honeydew—a sticky liquid in a closed gall may be harmful to the inhabitants—but still Pike et al. (2002) estimated that, at peak population size of *Pemphigus spyrothecae*, about 10 mm³ of honeydew is removed per gall per day! The quantity produced in galls of *Geoica wertheimae* and *B. pistaciae*, which house hundreds or even thousands of aphids in peak season, may be much greater. Moreover, waxed honeydew droplets, on which the aphids may walk (Inbar & Schulz, 2001), when pushed out of the galls (Kurosu & Aoki, 1991; Benton & Foster, 1992) may be less likely to evaporate and more likely to reach the forest floor. In our studies of the Fordinae in Israel, we searched

for colonies of the root-feeding forms of these species on secondary hosts: More often than not, the presence of ant nests around a batch of grasses led us to these colonies (D.Wool, O. Shukry, unpublished). Almost exclusively they were found a few meters away from gall-bearing *Pistacia* trees. In addition to "milking" feeding aphids within their nest, the honeydew released from the galls may be collected by ants as wax-packed food source. In the absence of quantitative data, this idea remains speculative.

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